Molecular mechanisms of Endothelial-Mesenchymal Transition in coronary artery stenosis and cardiac fibrosis

Vanchin, Byambasuren

Chapter 7

Research summary

RESEARCH SUMMARY

The ultimate goal of cardiovascular medicine is to reduce the global burden of cardiovascular disease and to improve the patients' quality-of-life. Fundamental to the development of successful medical treatments for cardiovascular diseases is an in-depth knowledge of the disease process, i.e. knowledge on the molecular signaling cascades in healthy cells and an in-depth understanding of how these molecular pathways get disturbed during disease. Endothelial homeostasis plays crucial role in vascular health by preventing unwarranted thrombosis, inflammation and regulating vascular tone. Endothelial dysfunction is a culprit in the development of many cardiovascular pathologies (1-3). Hence, insight into the molecular mechanisms that underlie endothelial dysfunction might allow for the discovery of innovative drugable targets to reduce the cardiovascular disease burden.

Adverse endothelial plasticity and its specialized form Endothelial-mesenchymal transition (EndMT) contributes to the development of multiple cardiovascular diseases including atherosclerosis (4, 5) and cardiac fibrosis (6). The pleiotropic cytokine TGFβ(7), inflammation (8) and oxidative stress (9) are established inducers of EndMT through the activation of canonical and non-canonical TGFβ signaling and the concurrent induction of mesenchymal gene expression by the transcription factors SNAIL, SLUG, TWIST1 and GATA4 (10, 11). The induction of gene expression is not only regulated by changes in transcription factor binding to the DNA, but also by the accessibility of the DNA to these transcription factors, which is tightly regulated by epigenetics. (**Chapter 1 and Chapter 2**) Epigenetic modifications, through changes in histone tail modifications and DNA methylation, facilitate the compaction and decompaction of the DNA, thereby orchestrating its accessibility to transcription factors. Furthermore, transcribed genes are not always translated into proteins, as non-coding RNAs might induce posttranscriptional inhibition. In **Chapter 2**, we proposed to therapeutically target epigenetic enzymes in the pro-atherogenic endothelium to ameliorate atherosclerosis development. We used the histone methyltransferase EZH2 and the histone deacetylase SIRT1 to exemplify how their pleiotropic functions can preclude atherosclerosis pathways and safeguard endothelial homeostasis.

In following chapters, we investigated the molecular mechanisms that drive EndMT with a focus on the multi-layered regulatory system consisting of epigenetic and post-transcriptional modifications. Furthermore, we investigated how these mechanisms contribute to the development of intimal hyperplasia and cardiac fibrosis.

ADVERSE ENDOTHELIAL PLASTICITY CONTRIBUTES TO THE DEVELOPMENT OF INTIMAL HYPERPLASIA

Intimal hyperplasia is an initiating event of atherosclerosis development and yet the molecular epigenetic mechanism is still elusive. Intimal hyperplasia is the consequence of migration and proliferation of fibro-proliferative cells in the tunica intima and which thickens the vascular wall (12). The origin of these fibro-proliferative cells is extensively investigated, and at least four types of cells are shown to give rise myofibroblast-like cells in the hyperplastic intima, i.e. resident fibroblasts, smooth muscle cells, endothelial cells and circulating progenitor cells. Vein grafting in an arterial environment by the

coronary artery bypass surgery (CABG) can trigger intimal hyperplasia and occlude the grafted vein (13), suggesting the importance of the EndMT in development of intimal hyperplasia. Also, endothelium-derived fibroproliferative cells accumulate in vascular areas exposed to low oscillatory shear stress where intimal hyperplasia is eminent (5) (14). Vice versa, vascular areas exposed to high laminar shear stress contain quiescent endothelial cells that are refractory to EndMT (14). Collectively, these data imply that fluid shear stress is a pivotal determinant of EndMT.

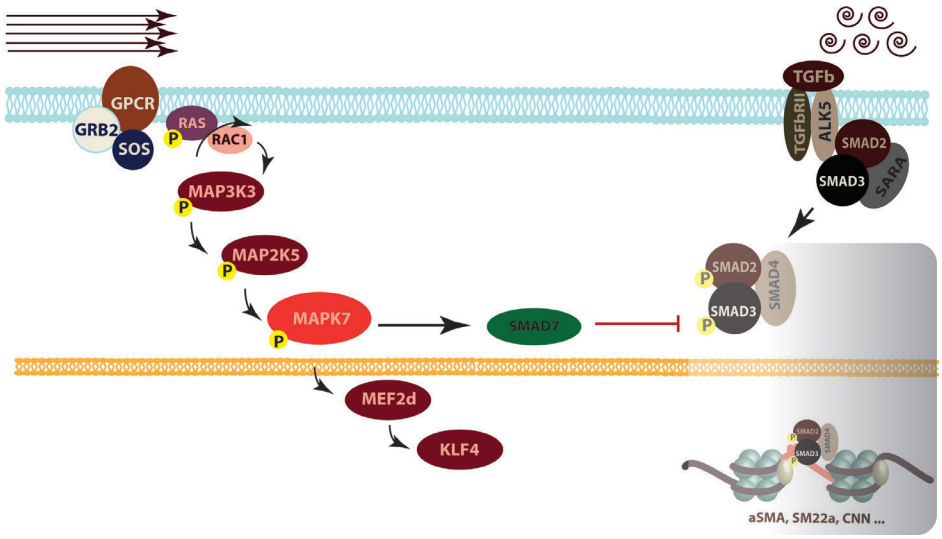

Figure 1. High laminar shear stress-induced MAPK7 activation precludes TGFβ-induced Endothelial-Mesenchymal transition. *Upon high laminar shear stress, endothelial MAPK7 signaling is activated. The downstream transcription factors of MAPK7, such as KLF4, are known to induce production of nitric oxide. The activation of MAPK7 signaling further results in the inhibition of TGFβ signaling by SMAD2/SMAD3, thereby inhibiting EndMT (14)*

In **Chapter 3**, we uncovered that at sites of intimal hyperplasia, MAPK7 expression and activity is silenced by microRNA-374b. MicroRNA-374b expression is induced by TGFβ and silences 4 target genes in the MAPK7 signaling cascade, i.e. MAP3K3, MAPK7, MEF2D and KLF4. Ectopic expression of microRNA-374b in endothelial cells, induces EndMT in the absence of exogenous TGFβ, suggesting that the microRNA-374b-dependent silencing of MAPK7 signaling is pivotal to intimal hyperplasia. Indeed, the expression of microRNA-374b is increased in experimental models of intimal hyperplasia where MAPK7 signaling is lost. (Figure 2). Moreover, in patients suffering from coronary artery disease, the expression of microRNA-374b associates with disease severity and with a decreased expression of MAPK7.

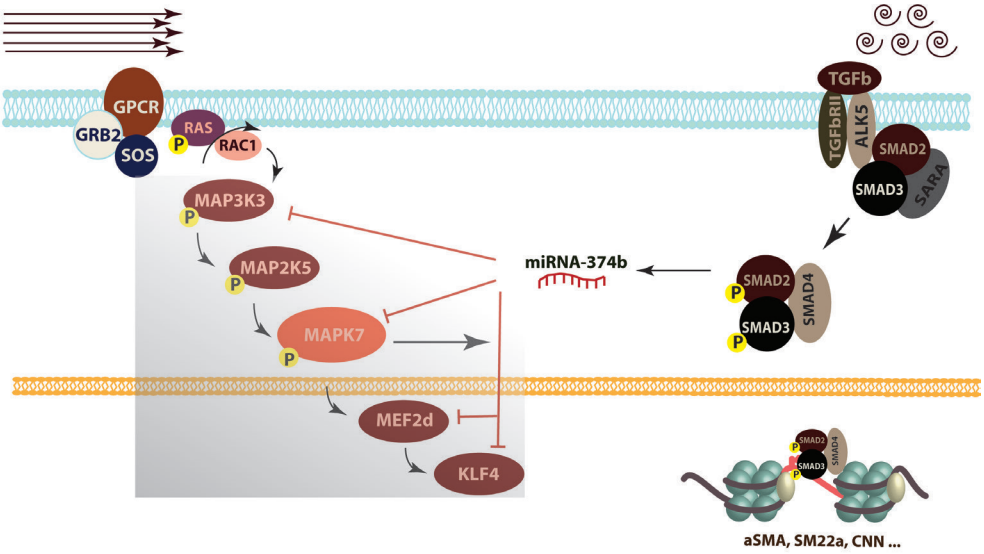

Figure 2. MicroRNA - 374b inhibits MAPK7 signaling and induces EndMT. *MicroRNA-374b is induced upon TGFβ1 stimulation and silences 4 members of the MAPK7 signaling cascade. This silencing mechanism explains how the MAPK7 signaling cascade is silenced by TGFβ in endothelial cells.*

Intimal hyperplasia is progressive process, which narrows the vascular lumen by the accumulation of fibroproliferative cells. How endothelium-derived fibroproliferative cells lose their quiescence and acquire this hyper-proliferative behavior is still elusive.

In **Chapter 4**, we uncovered that under atheroprotective - high laminar shear stress, the expression of the histone methyltransferase EZH2 is decreased. EZH2 is an epigenetic enzyme that tri-methylates lysine 27 on histone 3 (H3K27me3), which results in transcriptional repression (15). The increase in EZH2 is associated with elevated expression of cell cycle genes and increased proliferation of endothelial cells. It is still elusive how the reduction in EZH2 can result in a reduced cell cycling ability of endothelial cells, we conclude that the high laminar shear stress-dependent decrease in EZH2 expression facilitates endothelial quiescence (16). (Figure 3)

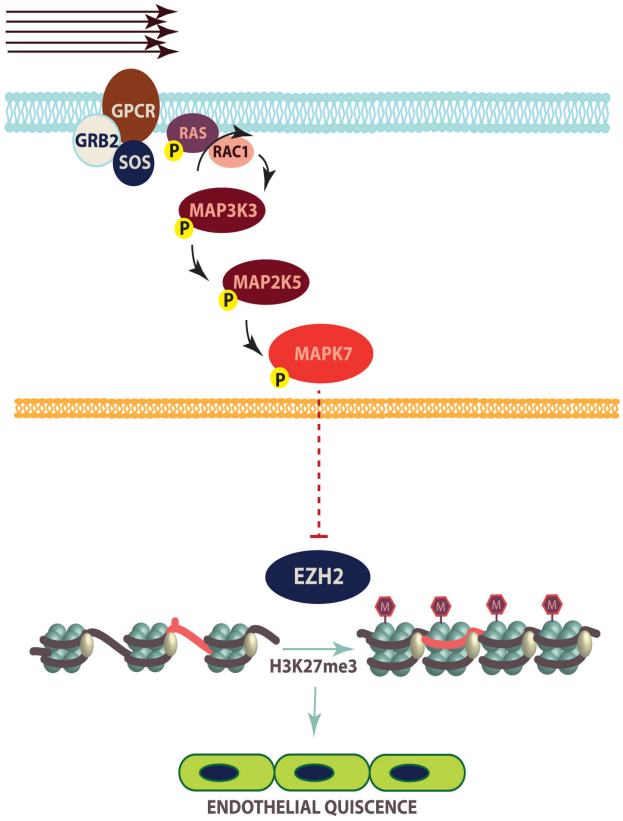

Figure 3. Uniform laminar shear stress mediated endothelial quiescence is mediated by the EZH2 decline. *Under the shear stress MAPK7 get phosphorylated and activated. This activation led to the decline in histone methyltransferase EZH2 protein level. RNA sequencing result revealed that both EZH2 decline and high laminar shear stress can inhibit cell cycle genes, which may explain to a certain extend how endothelial cells acquire endothelial quiescence.*

In Chapter 4, we noted that there was reciprocity between MAPK7 signaling and EZH2 expression, which cannot be explained by a direct interaction. Therefore, in **Chapter 5**, we investigated the reciprocity between MAPK7 and EZH2. Uniform laminar shear stress induces the MAPK7-dependent expression of microRNA-101 that silences EZH2 expression. Reciprocally, the loss of EZH2 expression results in the expression of microRNAs that target *DUSP1* and *6*, which inactivate MAPK7. In human coronary artery stenosis, *EZH2*, *DUSP1* and *DUSP6* level are increased whereas *MAPK7* expression is reduced. Moreover microRNA-101 expression is decreased which is associated with an increase in *EZH2* and the severity of the stenosis. These data might explain the difference in the development of intimal hyperplasia at sites exposed of high laminar shear stress and low oscillatory shear stress (Figure 4).

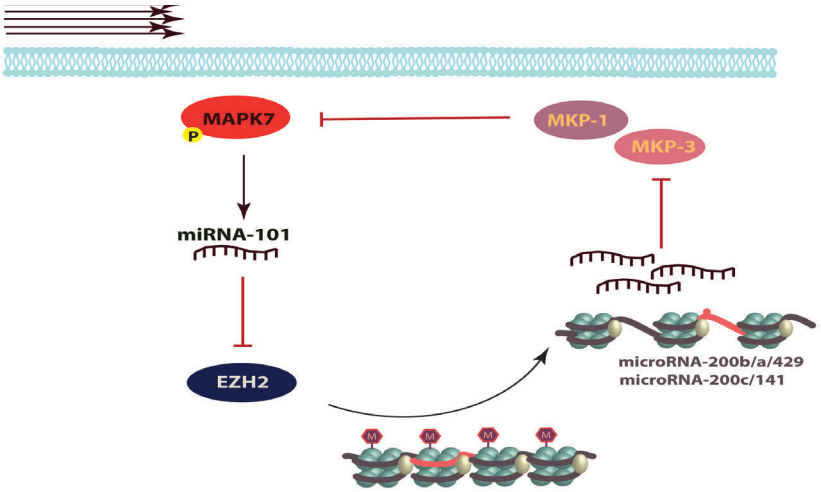

Figure 4. Reciprocal crosstalk between pMAPK7 and EZH2. Upon high laminar shear stress MAPK7 gets activated which induces the expression of miR-101. *Consequentially, the expression of EZH2 is silenced. On the other hand, EZH2 modulates the expression of miRNAs through H3k27me3 – a repressive chromatin mark. The decline of EZH2 leads to open chromatin, which elevates the expression level of these microRNAs. The microRNAs further silence the DUSP1 and DUSP6. This leads less protein expression of MKP-1 and MKP-3 which removes MAPK7 phosphorylation mark, thereby MAPK7 phosphorylation kept high.*

ADVERSE ENDOTHELIAL PLASTICITY IN CARDIAC FIBROSIS

Cardiac fibrosis is the fundamental pathology underlying heart failure for which no effective treatment is currently available. Plasma Galectin-3 levels are associated with all-cause mortality rate and recognized as a prognostic biomarker of cardiac fibrosis(17). GAL-3 knockdown attenuates cardiac fibrosis, indicating that Gal3 is not only a biomarker, but also a key molecule in the initiation of cardiac fibrosis (18). It has been reported that during cardiac fibrosis myofibroblast are derived from the endothelial lineage through EndMT(6). Hence, in **Chapter 6**, we investigated if Gal-3 could induce of EndMT. Although Gal-3 is generally believed to signal through a number of receptors, the addition of recombinant human Gal-3 did not induce or aggravate EndMT. In contrast, the loss of endogenous Gal-3 precluded TGFβ-induced EndMT via unknown mechanism. These data suggest that Gal-3 might act as a transcriptional co-activation during TGFβ-induced EndMT (19, 20), which is currently under investigation

CONCLUSIONS

Endothelial-mesenchymal transition is a crucial component of atherosclerosis and cardiac fibrosis development and shown to be modulated by fluid shear stress. In this thesis, we show that endothelial-mesenchymal transition is regulated on multiple

levels by MAPK7, EZH2 and microRNAs and found dysregulation of these factors in experimental models of intimal hyperplasia and in human coronary artery disease. We uncovered a number of pivotal regulators of EndMT that might have therapeutic potential to ameliorate intimal hyperplasia and cardiac fibrosis.

REFERENCES

1. Gimbrone MA, Topper JN, Nagel T, Anderson KR, GARCIA-CARDEÑA G. Endothelial dysfunction, hemodynamic forces, and atherogenesis. Annals of the New York Academy of Sciences. 2000;902(1):230-40.
2. Sena CM, Pereira AM, Seiça R. Endothelial dysfunction—a major mediator of diabetic vascular disease. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2013;1832(12):2216-31.
3. Vanhoutte P, Shimokawa H, Feletou M, Tang E. Endothelial dysfunction and vascular disease–a 30th anniversary update. Acta Physiologica. 2017;219(1):22-96.
4. Chen P-Y, Qin L, Baeyens N, Li G, Afolabi T, Budatha M, et al. Endothelial-to-mesenchymal transition drives atherosclerosis progression. The Journal of clinical investigation. 2015;125(12):4514.
5. Evrard SM, Lecce L, Michelis KC, Nomura-Kitabayashi A, Pandey G, Purushothaman K-R, et al. Endothelial to mesenchymal transition is common in atherosclerotic lesions and is associated with plaque instability. Nature communications. 2016;7.
6. Zeisberg EM, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, Gustafsson E, et al. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. Nature medicine. 2007;13(8):952.
7. Arciniegas E, Sutton AB, Allen TD, Schor AM. Transforming growth factor beta 1 promotes the differentiation of endothelial cells into smooth muscle-like cells in vitro. Journal of cell science. 1992;103(2):521-9.
8. Maleszewska M, Moonen J-RA, Huijkman N, van de Sluis B, Krenning G, Harmsen MC. IL-1β and TGFβ2 synergistically induce endothelial to mesenchymal transition in an NFκB-dependent manner. Immunobiology. 2013;218(4):443-54.
9. Lee ES, Boldo LS, Fernandez BO, Feelisch M, Harmsen MC. Suppression of TAK1 pathway by shear stress counteracts the inflammatory endothelial cell phenotype induced by oxidative stress and TGF-β1. Scientific Reports. 2017;7:42487.
10. Souihol CE, Harmsen MC, Evans PC, Krenning G. Endothelial-Mesenchymal Transition in Atherosclerosis. Cardiovascular research. 2018.
11. Medici D, Potenta S, Kalluri R. Transforming growth factor-β2 promotes Snail-mediated endothelial–mesenchymal transition through convergence of Smad-dependent and Smad-independent signalling. Biochemical Journal. 2011;437(3):515-20.
12. Ross R, Glomset JA. Atherosclerosis and the arterial smooth muscle cell. Science. 1973;180(4093):1332-9.
13. Cooley BC, Nevado J, Mellad J, Yang D, Hilaire CS, Negro A, et al. TGF-β signaling mediates endothelial-to-mesenchymal transition (EndMT) during vein graft remodeling. Science translational medicine. 2014;6(227):227ra34-ra34.
14. Moonen J-RA, Lee ES, Schmidt M, Maleszewska M, Koerts JA, Brouwer LA, et al. Endothelial-to-mesenchymal transition contributes to fibro-proliferative vascular disease and is modulated by fluid shear stress. Cardiovascular research. 2015;108(3):377-86.
15. Di Croce L, Helin K. Transcriptional regulation by Polycomb group proteins. Nature structural & molecular biology. 2013;20(10):1147-55.
16. Maleszewska M, Vanchin B, Harmsen MC, Krenning G. The decrease in histone methyltransferase EZH2 in response to fluid shear stress alters endothelial gene expression and promotes quiescence. Angiogenesis. 2016;19(1):9-24.
17. Ho JE, Liu C, Lyass A, Courchesne P, Pencina MJ, Vasan RS, et al. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. Journal of the American College of Cardiology. 2012;60(14):1249-56.

18. Yu L, Ruifrok WP, Meissner M, Bos EM, van Goor H, Sanjabi B, et al. Genetic and pharmacological inhibition of galectin-3 prevents cardiac remodeling by interfering with myocardial fibrogenesis. Circulation: Heart Failure. 2012:CIRCHEARTFAILURE. 112.971168.
19. MacKinnon AC, Gibbons MA, Farnworth SL, Leffler H, Nilsson UJ, Delaine T, et al. Regulation of transforming growth factor-β1–driven lung fibrosis by galectin-3. American journal of respiratory and critical care medicine. 2012;185(5):537-46.
20. Shimura T, Takenaka Y, Tsutsumi S, Hogan V, Kikuchi A, Raz A. Galectin-3, a novel binding partner of β-catenin. Cancer research. 2004;64(18):6363-7.